Effect of Abraded Intramammary Device on Milk Yield, Tissue Damage, and Cellular Composition[1]

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ABSTRACT

The study was conducted to determine effects of an abraded intramammary device on milk yield, tissue damage, and milk somatic cells during late lactation. Abraded intramammary devices were inserted into diagonally opposed quarters of 20 cows. One group of 11 cows (devices inserted 30 wk of lactation) was quarter machine milked daily for 2 wk before and for 8 wk after insertion. Nine cows had devices inserted within 4 wk postpartum and were quarter milked at 20 and 40 wk of lactation. Aliquots of quarter bucket milk were obtained for determination of milk somatic cells, red blood cells, and NAGase activity. Stripping milk was obtained for milk somatic cell counts. For both groups, there was no significant effect of the abraded intramammary device on NAGase activity. There was a tendency for quarters with devices to produce less milk than control quarters, but the difference was not significant. Milk SCC were significantly elevated in quarter bucket and stripping milk from quarters containing intramammary devices. Red blood cells were higher in quarters containing intramammary devices when compared with controls. Results indicate that abraded intramammary devices increased concentrations of somatic cells and red blood cells in bucket milk, did not damage tissue as measured by NAGase activity, and loss in milk production was nonsignificant.

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INTRODUCTION

Although results from experimental and field studies (1, 18, 21, 22, 27, 28) indicate that an intramammary device (IMD) placed into the mammary gland cistern of cows stimulates intramammary defense mechanisms for protection, several concerns have surfaced. Concentration of somatic cells in bucket milk from cows in early to midlactation increases slightly after IMD placement (18). Effects during late lactation are not known. Milk somatic cell counts (MSCC) are routinely determined to ensure that commercial milk is in compliance with the federal standards for Grade A raw milk (5). Furthermore, purchasers of commercial milk will commonly pay a premium for low MSCC. On large commercial dairies, this premium could amount to an additional $500/mo (W. Stouder, 1986, personal communication). Emphasis on MSCC as an indicator of milk quality is understandable, because MSCC increases in response to mastitis, which in turn is associated with changes in concentrations of nearly all major and minor constituents of milk (24). Because of these concerns, development of an IMD that elevates MSCC in stripping milk should be carried out in conjunction with an evaluation of its effects on MSCC in bucket milk at various stages of lactation.

Increased MSCC is associated with decreased milk yield (8). The decrease is thought to be attributable to damage of mammary secretory cells by leukocytes (2). Effects of IMD on damage to secretory cells are not known. N-Acetyl-β-D-glucosaminidase (EC 3.2.1.30) is used as a marker of epithelial cell damage (9). Elevations of NAGase in milk from abraded IMD (AIMD) quarters compared with NAGase in control quarters may provide information on damage to mammary epithelial cells.

Effects of AIMD on milk production indicate that insertion of AIMD in cows increases milk production (13). The increased milk yield

is attributable to decreased incidence of clinical mastitis in cows with AIMD (28). Effects on milk production in udders that remain uninfected is less clear. Two studies (6, 21) reported no significant effect of IMD on milk yield, but Jaster et al. (7) found that IMD quarters produced .6 kg less milk per quarter per d. Huston and Heald (6), after adjusting their data to full daily milk yield, found that the loss approximated 1 kg of milk per cow per d. They found the milk loss to be equivalent to the loss reported for a similar increase in MSCC in a study (8) based on data from 43,677 cows. More information on effects of IMD in uninfected udders is needed.

In studies at Beltsville with earlier IMD prototypes, blood was observed in milk from quarters with IMD but not in milk from control quarters (1, 18). Bleeding was attributed to breakage of the IMD in situ and to damage of the teat caused by the large diameters of the cannula (3.7 mm) used to insert the IMD, which was 2.5 mm in diameter. Breakage of the IMD was attributed to the molding process used, which left weak areas in the IMD. Breakage was eliminated by using IMD made with an extrusion process. Examination of over 200 extruded IMD removed from live cows or recovered at the time of slaughter indicated no broken IMD (10, 28). Reducing the size of the cannula and IMD to 2.4 mm and 1.5 mm, respectively, eliminated bleeding caused by trauma to the teat end (28). Because appearance of red blood cells (RBC) in milk may suggest an abnormal condition, a minimal RBC response is desired to an IMD used prophylactically.

The purpose of the present study was to investigate effects of AIMD on milk yield, on MSCC in stripping and primary milk, and on concentration of RBC and NAGase activity in primary milk.

MATERIALS AND METHODS

Abraded IMD, measuring 1.5 mm in diameter and 120 mm in length and forming a loop of 25 mm in diameter, were inserted into diagonally opposed quarters of 20 cows. Devices were abraded with medium grade emery cloth. All quarters were free from infection. One group of 11 cows (214 d of lactation) was quarter machine milked daily for 2 wk before and for 8 wk after insertion of devices. Nine cows had AIMD inserted within 4 wk postpartum and were quarter machine milked at 20 and 40 wk of lactation. This second group of cows was used in an earlier *Escherchia coli* challenge study (1). Cows were challenged 4 wk after AIMD insertion according to previously described methods (1). All control quarters and 38% of the AIMD quarters became infected. Infections were eliminated after antibiotic therapy for four consecutive milkings, beginning on the 10th d after bacterial challenge.

Quarter milk weights were recorded at every milking. Aliquots of quarter bucket milk and stripping milk samples were obtained immediately after milking. Quarter bucket milk samples were assayed for MSCC, RBC concentration, and NAGase activity. Stripping milk was assayed for MSCC. The MSCC were determined on a Coulter electronic cell counter (16). Cytospin smears were prepared for semiquantitation of RBC. Briefly, 1 ml milk was added to 8 ml phosphate-buffered (.0132 *M*, pH 7.4) .85% sodium chloride (PBSS) containing 5% normal bovine serum in a conical shaped graduated centrifuge tube and centrifuged at 175 × *g* (10 min, 5°C). Cream and supernatant were aspirated leaving exactly 1 ml. Tubes were vortexed to resuspend cells, and a .2-ml sample was added to cytospin chamber (Shandon-Southern Cytospin (SCA-0030) 93-96 Chadwick Rd., Astmoor Industrial Estate, Runcorn, Cheshire, England WA 71PR), which was capped and spun for 10 min at 225 × *g*. Smears were air dried and stained with Wright's stain. Smears were examined under oil immersion using the single count method and wide reticle (15). Milk NAGase activity was determined using the procedure of Kitchen et al. (9) as modified by Dulin et al. (3). Weekly foremilk samples were collected aseptically for diagnostic bacteriology (14).

For statistical analysis, MSCC were transformed to $\log_{10}$. Analysis of variance was conducted to determine significance in MSCC, NAGase, RBC, and milk yield between control and abraded IMD-treated mammary quarters by time of sampling relative to IMD insertion. Duncan's multiple range test was used to assess individual differences among treatment means.

TABLE 1. Effect of abraded intramammary device (AIMD) on quarter bucket milk weights.

Time relative to insertion	Quarters		Significance
	Control	AIMD	
(wk)	(kg/milking)		
Group 1 (11 cows)[1]			
−2	2.6[a]	2.6[a]	NS[2]
4	2.6[a]	2.4[a]	NS
8	1.8[b]	1.6[b]	NS
Group 2 (9 cows)[3]			
20	2.6[a]	2.5[a]	NS
40	2.1[b]	1.8[b]	NS

[a,b]Means in a column, within a group, with different superscripts differ ($P<.05$).

[1] The AIMD was inserted 30 wk after calving.

[2] NS = Not significant.

[3] The AIMD was inserted within 4 wk after calving.

RESULTS

Quarter milk yield for control and AIMD quarters is shown (Table 1). Milk yield decreased ($P<.05$) with increasing stage of lactation for both groups. For group 1, after AIMD insertion, control quarters averaged .2 kg more milk at each milking than did AIMD quarters. The difference, however, was not significant ($P>.05$). A greater difference in milk yield was apparent in the second group of cows. By the 40th wk of lactation, 36 wk after AIMD insertion, control quarters were averaging .3 kg more milk at each milking than were AIMD quarters. Again, the difference was not significant. The MSCC in both quarter bucket and stripping milk increased ($P<.05$) with increasing stage of lactation (Table 2). For the first group of cows, quarter bucket MSCC for control and AIMD quarters increased 1.3 and 2.6 times from 2 wk before insertion to 8 wk after

TABLE 2. Milk somatic cell count (geometric means) in quarter bucket and stripping milk from control and abraded intramammary deviced (AIMD) quarters.

Time relative to insertion	Quarters			Quarters		
	Control	AIMD	Significance	Control	AIMD	Significance
(wk)	($\times 10^6$/ml bucket milk)			($\times 10^6$/ml stripping milk)		
Group 1 (11 cows)[1]						
−2	.150[a]	.156[a]	NS[2]	.325[a]	.321[a]	NS
4	.103[b]	.222[a]	$P<.05$	.227[a]	.728[b]	$P<.05$
8	.196[a]	.400[b]	$P<.05$	.529[b]	1.156[c]	$P<.05$
Group 2 (9 cows)[3]						
20	.090[a]	.218[a]	$P<.01$	.129[a]	.387[a]	$P<.01$
40	.156[b]	.488[b]	$P<.01$	.318[b]	1.005[b]	$P<.01$

[a,b]Means in a column, within a group, with different superscripts differ ($P<.05$).

[1] The AIMD was inserted 30 wk after calving.

[2] NS = Not significant.

[3] The AIMD was inserted within 4 wk after calving.

TABLE 3. Concentration of red blood cells in quarter bucket milk from control and abraded intramammary deviced (AIMD) quarters.

Time relative to insertion	Quarters		Significance
	Control	AIMD	
(wk)	(×10⁶/ml)		
Group 1 (11 cows)[1]			
−2	.07[a]	.11[a]	NS[2]
4	.20[a]	23.02[b]	$P<.05$
8	.00[a]	27.78[b]	$P<.05$
Group 2 (9 cows)[3]			
20	.98[a]	11.36[a]	NS
40	1.51[a]	47.28[a]	$P<.05$

[a,b]Means in a column, within a group, with different superscripts differ ($P<.05$).

[1] The AIMD was inserted 30 wk after calving.

[2] NS = Not significant.

[3] The AIMD was inserted within 4 wk after calving.

insertion. For stripping milk the increase was 1.6 and 3.6 times. For the second group, quarter bucket MSCC for control and AIMD quarters increased 1.7 and 2.2 times between 20 and 40 wk. The MSCC in stripping milk increased 2.4 and 2.6 times. After AIMD insertion, quarter bucket MSCC were higher ($P<.05$) in AIMD quarters than in control quarters for both groups. For the first group, by 4 wk after insertion, the difference was 2.2 times and at 8 wk it was 2.0 times greater. For the second group, the difference at 20 wk after insertion was 2.4 times and by 40 wk it was 3.1 times greater.

Concentration of RBC in quarter bucket milk did not increase with stage of lactation for either control or AIMD quarters (Table 3). Interestingly, RBC were routinely observed in

TABLE 4. N-Acetyl-β-D-glucosaminidase activity in quarter bucket milk from control and abraded intramammary deviced (AIMD) quarters.

Time relative to insertion	Quarters		Significance
	Control	AIMD	
(wk)	(μmol/min per ml)		
Group 1 (11 cows)[1]			
−2	.014	.015[a]	NS[2]
4	.013	.015[a]	NS
8	.018	.025[b]	$P<.05$
Group 2 (9 cows)[3]			
20	.006[a]	.014	NS
40	.013[b]	.022	NS

[a,b]Means in a column, within a group, with different superscripts differ ($P<.05$).

[1] The AIMD was inserted 30 wk after calving.

[2] NS = Not significant.

[3] The AIMD was inserted within 4 wk after calving.

bucket milk from control quarters. Greatest concentrations in control quarters were observed in group 2 cows at 44 wk of lactation, which averaged 1.51 x 10^6/ml. After AIMD insertion, concentrations were consistently higher in AIMD quarters than in control quarters. Blood was occasionally observed visually in stripping milk but never in bucket milk.

Milk NAGase activity tended to increase with stage of lactation; the increase was significant for group 1 AIMD quarters and for group 2 control quarters (Table 4). There was also a tendency for NAGase activity to be higher for AIMD quarters when compared to control quarters. The difference was significant only for group 1 cows at 8 wk after insertion.

DISCUSSION

Results from this study suggest that uninfected mammary quarters with AIMD produce less milk. There appeared to be a consistent depression in milk yield from AIMD quarters, but because of the variability in daily quarter milk weights, the decrease was not significant. The results support previous studies, where depressions in milk yield of the same magnitude as in the present study (.1 to .3 kg/milking) were observed (6, 7, 21). In only one of those studies was the decrease significant (7). It appears that quarter milk yield is too variable to evaluate effects of the IMD on milk production with small numbers of cows. Comparison between IMD and control cows in field studies, involving large numbers of animals, appears to be the more appropriate experimental design to use. Preliminary results from one such study, which is evaluating effects of AIMD on milk production from approximately 6000 Israeli dairy cows, indicate that cows with AIMD produce 1.9 kg more milk/d than controls (13). However, the gain in milk production in cows with AIMD appears to be due to reduced clinical mastitis in those cows (28). Differences between AIMD and control cows that never had mastitis have yet to be determined.

The significant increase in MSCC in bucket milk from AIMD quarters agrees with results from previous studies (18, 20, 21). The largest increase in quarter MSCC occurred in the group of cows with the longest lactation (approximately 290 d). Here, AIMD quarters averaged .332 x 10^6 more cells/ml than did control quarters. These data suggest that as milk volume in the gland decreases, chemotactic factors generated by the AIMD become more heavily concentrated in the milk, causing infiltration of leukocytes further up in the gland and into primary milk. However, it should be noted that a similar, although less pronounced, increase in MSCC occurred in control quarters as lactation advanced.

The increase in MSCC in stripping milk from AIMD quarters agrees with previous data (1, 18). Ziv et al. (27), in a study with uninfected primiparous cows, reported that the proportion of quarters having MSCC greater than one million in stripping milk gradually decreased from 95.3% of the quarters at 2 wk after AIMD insertion to 36.6% by 6 mo. Those results suggest that the AIMD gradually loses its ability to maintain the protective concentration of 900,000 cells/ml of stripping milk needed for protection against intramammary infection (25). Results from the present study do not support the gradual decrease in MSCC with advancing lactation. In group 2 cows, where AIMD were in place for approximately 42 wk, MSCC in stripping milk averaged 1.005 x 10^6/ml.

Several factors could be responsible for the appearance of RBC in quarter bucket milk. Their presence could be attributable to an abrasive effect of the AIMD to the epithelial lining of the gland cistern. Huston and Heald (6), in studies with a smooth IMD, reported a significant change toward a single layer of epithelial cells in gland cisterns of IMD quarters when compared to controls. The change to a single layer of cells may allow for a greater opportunity for the AIMD to disturb the integrity of the glandular epithelium, thus allowing for possible hemorrhaging to occur. The appearance of RBC in milk is probably not related to possible disruption of the glandular epithelium caused by increased movement of leukocytes into milk. Studies using bovine and rabbit granulocytes, indicate that migration of granulocytes across intact, viable endothelium in response to chemotactic agents does not result in structural injury to the endothelium (17, 27).

The appearance of RBC in milk may be related to the release of digestive enzymes and toxic oxygen products from phagocytic leukocytes as a consequence of ingestion of milk fat

globules and casein (19, 23). These products contribute toward disruption of both the vascular endothelium and glandular epithelium (2, 11). The high concentration of RBC in group 2 control cows could be a carryover effect as a result of those quarters having been previously infected with *E. coli*. There is no information in the literature concerning concentrations of RBC in milk from uninfected or infected quarters or on the effects of prior infection on concentration of RBC in milk.

Milk NAGase activity, as an index of AIMD-induced tissue damage, must be interpreted cautiously. In all but one of the groups, there was no significant difference in NAGase activity between control and AIMD quarters. The NAGase is contained within lysosomes of epithelial cells, neutrophils, and macrophages (4, 9, 12), and the release of this enzyme from white blood cells contributes to NAGase activity in milk (3). This makes evaluation of the amount released from mammary epithelial cells difficult. For example, for group 1 cows there was a significant difference in NAGase activity between control and AIMD quarters. Quarters with AIMD averaged $.204 \times 10^6$ more somatic cells and .007 μmol/min per ml more NAGase than control quarters. Using regression equations derived by Dulin et al. (3), which determines amount of NAGase contributed by milk leukocytes ($.204 \times 10^6$ somatic cells contribute .007 μmol/min per ml of NAGase), the entire increase in NAGase activity in AIMD quarters can be accounted by the increased MSCC. These data indicate that AIMD will not cause release of NAGase from mammary epithelial cells (an indicator of epithelial cell damage).

CONCLUSIONS

Results indicate a tendency for AIMD quarters to produce .1 to .3 kg less milk per milking than control quarters. The lack of any difference in NAGase activity (an indirect measure of mammary epithelial cell damage) between AIMD and control quarters indicated no damage to milk secretory tissue. However, the microscopic presence of RBC in quarter bucket milk from AIMD quarters suggested some local hemorrhaging in the gland cistern as a consequence of the AIMD. Milk SCC in bucket milk from AIMD quarters approached 500,000/ml and was 3.3 times higher than control quarters. The elevated cell counts in AIMD quarters could be of economic importance in herds with large numbers of cows in late lactation, thereby possibly elevating bulk tank MSCC.

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